

AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions and listings of claims in the application:

LISTING OF CLAIMS:

1. (previously presented): A mutant Bcl-X_L/Bcl-2 Associated Cell Death Regulator polypeptide (BAD), wherein said mutant BAD:

a) has an amino acid sequence which is at least 95% homologous to the amino acid sequence of SEQ ID NO:1;

b) has an amino acid substitution at the position corresponding to position 118 of SEQ ID NO:1, wherein said amino acid is alanine or an amino acid conservative for alanine; and

c) has cell death promoting activity *in vitro*; or
a fragment of said mutant BAD, wherein said fragment comprises the BH3 domain of said mutant BAD and has cell death promoting activity *in vitro*.

2. (canceled).

3. (currently amended): The mutant BAD of Claim 1, wherein the amino acid sequence of said mutant BAD is identical to SEQ ID NO:1, with the proviso that the amino acid ~~at the position corresponding to position 118 of SEQ ID NO:1~~ is alanine or an amino acid conservative for alanine.

4-9. (canceled).

10. (previously presented): The mutant BAD or fragment of mutant BAD of Claim 1, wherein said mutant BAD or said fragment binds Bcl-X_L or Bcl-2, or both.

11-15. (canceled).

16. (previously presented): The mutant BAD or fragment of mutant BAD of Claim 1, wherein the amino acid at the position corresponding to position 118 of SEQ ID NO:1 is alanine.

17-18. (canceled).

19. (previously presented): The mutant BAD or fragment of mutant BAD of Claim 1, wherein the amino acid conservative for alanine at the position corresponding to position 118 of SEQ ID NO:1 is an amino acid other than glycine.

20-21. (canceled).

22. (previously presented): The mutant BAD or fragment of mutant BAD of Claim 1, wherein the amino acid at the position corresponding to position 118 of SEQ ID NO:1 is not alanine.

23-24. (canceled).

25. (previously presented): The mutant BAD or fragment of mutant BAD of Claim 1, wherein said mutant BAD or said fragment comprises an amino acid sequence corresponding to positions 103-123 of SEQ ID NO:1, with the proviso that the amino acid at the position corresponding to position 118 of SEQ ID NO:1 is alanine or an amino acid conservative for alanine.

26-30. (canceled).

31. (original) A method for making a mutant Bcl-X_L/Bcl-2 Associated Cell Death Regulator polypeptide (BAD) comprising an amino acid sequence of a naturally-occurring or wild-type mammalian BAD, or fragment of said mutant BAD comprising a less than full-length amino acid sequence of said naturally-occurring or wild-type mammalian BAD, said method comprising:

a) selecting an amino acid sequence of a naturally-occurring or wild-type mammalian BAD, or selecting a less than full-length amino acid sequence of said naturally-occurring or wild-type mammalian BAD, comprising a BH3 domain substantially identical to the BH3 domain encoded by the amino acids at positions 114-122 of SEQ ID NO:1, positions 151-159 of SEQ ID NO:2, or positions 109-117 of SEQ ID NO:3, said BH3 domain of said naturally-occurring or wild-type mammalian BAD, or said BH3 domain of said fragment of said naturally-occurring or wild-type mammalian BAD, being identified by alignment of said amino acid sequence of said naturally-occurring or wild-type mammalian BAD, or said amino acid sequence of said fragment of said naturally-occurring or wild-type mammalian BAD, to SEQ ID NO:1, SEQ ID NO:2, or SEQ ID NO:3, respectively; and

b) changing the amino acid of said amino acid sequence of said naturally-occurring or wild-type mammalian BAD, or said amino acid sequence of said fragment of said naturally-occurring or wild-type mammalian BAD, at a position corresponding to position 118 of SEQ ID NO:1, position 155 of SEQ ID NO:2, or position 113 of SEQ ID NO:3 to an amino acid other than serine, thereby, making:

1) said mutant BAD comprising said amino acid sequence of said naturally-occurring or wild-type mammalian BAD having a mutation at said amino acid, or

2) said fragment of mutant BAD comprising said amino acid sequence that is a less than full-length amino sequence of said naturally-occurring or wild-type mammalian BAD having a mutation at said amino acid, respectively.

32. (original) The method of Claim 31, wherein the amino acid at said position corresponding to position 118 of SEQ ID NO:1, position 155 of SEQ ID NO:2, or position 113 of SEQ ID NO:3 is alanine.

33. (original) The method of Claim 31, wherein said amino acid sequence of said naturally-occurring or wild-type mammalian Bcl-X_L/Bcl-2 Associated Cell Death Regulator polypeptide (BAD) is SEQ ID NO:1, SEQ ID NO:2, or SEQ ID NO:3, or said amino acid sequence of said fragment of said naturally-occurring mammalian or wild-type mammalian BAD is a less than full-length amino acid sequence of SEQ ID NO:1, SEQ ID NO:2, or SEQ ID NO:3.

34. (original) The method of Claim 31, further comprising expressing said mutant Bcl-X_L/Bcl-2 Associated Cell Death Regulator polypeptide (BAD), or said fragment of said mutant BAD, in a host cell, wherein said host cell is transformed with a polynucleotide comprising said amino acid sequence of said mutant BAD, or said host cell is transformed with a polynucleotide comprising said amino acid sequence of said fragment of said mutant BAD, respectively.

35. (original) A method of screening a candidate drug for activity that promotes apoptosis, said method comprising:

a) contacting a candidate drug with a sample comprising a mammalian Bcl-X_L/Bcl-2 Associated Cell Death Regulator polypeptide (BAD), or fragment of said mammalian BAD, and a kinase, to form a reacted fraction,

1) said mammalian BAD, or said fragment, comprising an amino acid sequence containing a serine at a position corresponding to position 118 of SEQ ID NO:1, position 155 of SEQ ID NO:2, or position 113 of SEQ ID NO:3, said position of said serine being identified by alignment of said amino acid sequence of said mammalian BAD, or said amino acid sequence of said fragment, to SEQ ID NO:1, SEQ ID NO:2, or SEQ ID NO:3, respectively,

2) said kinase having phosphorylation activity capable of phosphorylating said mammalian BAD; and

b) comparing said reacted fraction to a control fraction, to determine whether said candidate drug inhibits said phosphorylation activity of said kinase and, thereby, has activity that promotes apoptosis, by assaying for an amount of said mammalian BAD, or said fragment, that is unphosphorylated at said serine in said reacted fraction as compared to said control fraction.

36. (original) The method of Claim 35, wherein said assaying includes assaying for an amount of mammalian BAD, or said fragment, that is bound to Bcl-X_L and/or Bcl-2 in said isolated fraction as compared to said control fraction.

37. (original) A method of inducing apoptosis in a cell expressing a mammalian Bcl-X_L/Bcl-2 Associated Cell Death Regulator polypeptide (BAD), or fragment of said mammalian BAD, said method comprising:

a) preparing a culture containing a cell line expressing said mammalian BAD, or said fragment,

1) said mammalian BAD, or said fragment, comprising an amino acid sequence containing a serine at a position corresponding to position 118 of SEQ ID NO:1, position 155 of SEQ ID NO:2, or position 113 of SEQ ID NO:3, said position of said serine being identified by alignment of said amino acid sequence of said mammalian BAD, or said amino acid sequence of said fragment, to SEQ ID NO:1, SEQ ID NO:2, or SEQ ID NO:3, respectively;

b) contacting said cultured cells with an extracellular agent, and/or inducing an intracellular agent, capable of inhibiting the phosphorylation activity of a kinase in said cell, said kinase activity capable of phosphorylating said serine, to form a reacted fraction; or

c) contacting said cultured cells with an extracellular agent, and/or inducing an intracellular agent, capable of activating the phosphatase activity of a phosphatase in said cell capable of dephosphorylating said mammalian BAD, or said fragment, that is phosphorylated at said serine, to form a reacted fraction; and

d) comparing the cells in said reacted fraction to control cells to determine whether apoptosis is induced in the cells in said reacted fraction by,

1) assaying for an amount of said mammalian BAD, or said fragment, that is unphosphorylated and/or dephosphorylated in the cells in said reacted fraction as compared to said control cells, or

2) monitoring indicia of apoptosis in the cells in said reacted fraction as compared to said control cells.

38. (original) The method of Claim 37, wherein said kinase is the cyclic AMP (cAMP)-dependent protein kinase, PKA.

39. (original) The method of Claim 37, wherein said inhibiting is carried out by inhibitor H89, wherein said H89 inhibits the phosphorylation activity of said kinase.

40. (original) The method of Claim 37, wherein said inhibiting is carried out by:

- a) the binding of a polypeptide or a polynucleotide to said kinase, and thereby inhibiting the phosphorylation activity of said kinase; or
- b) binding of a polypeptide or polynucleotide to a polynucleotide that encodes said kinase, preventing the expression of said kinase, and thereby inhibiting the phosphorylation activity of said kinase.

41. (original) A method of assaying a candidate compound for phosphatase activity capable of dephosphorylating a mammalian Bcl-X_L/Bcl-2 Associated Cell Death Regulator polypeptide (BAD), or fragment of said mammalian BAD, at a serine at a position in the amino acid sequence of said mammalian BAD, or the amino acid sequence of said fragment, corresponding to position 118 of SEQ ID NO:1, position 155 of SEQ ID NO:2, or position 113 of SEQ ID NO:3, said position of said serine being identified by alignment of said amino acid sequence of said mammalian BAD, or said amino acid sequence of said fragment, to SEQ ID NO:1, SEQ ID NO:2, or SEQ ID NO:3, respectively, said method comprising:

- a) contacting said candidate compound with said mammalian BAD, or said fragment, to form a reacted fraction, wherein said mammalian BAD, or said fragment, is dephosphorylated at said serine; and

b) comparing said reacted fraction to a control fraction to determine whether said candidate compound has said phosphatase activity by assaying for an amount of said mammalian BAD, or said fragment, that is bound to Bcl-X_L and/or Bcl-2 in said reacted fraction as compared to said control fraction.

42. (original) A method of assaying a candidate compound for phosphatase activity capable of dephosphorylating a mammalian Bcl-X_L/Bcl-2 Associated Cell Death Regulator polypeptide (BAD), or fragment of said mammalian BAD, at a serine at a position in the amino acid sequence of said mammalian BAD, or the amino acid sequence of said fragment, corresponding to position 118 of SEQ ID NO:1, position 155 of SEQ ID NO:2, or position 113 of SEQ ID NO:3, said position of said serine being identified by alignment of said amino acid sequence of said mammalian BAD, or said amino acid sequence of said fragment, to SEQ ID NO:1, SEQ ID NO:2, or SEQ ID NO:3, respectively, said method comprising:

a) contacting said candidate compound with said mammalian BAD, or said fragment, to form a reacted fraction, wherein said mammalian BAD, or said fragment, is dephosphorylated at said serine, or capable of being de-phosphorylated at said serine; and

b) comparing said reacted fraction to a control fraction to determine whether said candidate compound has said phosphatase activity by assaying for an amount of said mammalian BAD, or said fragment, that is dephosphorylated at said serine in said reacted fraction as compared to said control fraction.

43. (original) A method of screening a candidate drug for activity that promotes cell survival, said method comprising:

a) contacting said candidate drug with a mammalian Bcl-X_L/Bcl-2 Associated Cell Death Regulator polypeptide (BAD), or fragment of said mammalian BAD, and, optionally, a kinase, to form a reacted fraction,

1) said mammalian BAD, or said fragment, capable of being phosphorylated by said kinase at a serine at a position in the amino acid sequence of said mammalian BAD, or amino acid sequence of said fragment, corresponding to position 118 of SEQ ID NO:1, position 155 of SEQ ID NO:2, or position 113 of SEQ ID NO:3, said position of said serine being identified by alignment of said amino acid sequence of said mammalian BAD, or amino acid sequence of said fragment, to SEQ ID NO:1, SEQ ID NO:2 and SEQ ID NO:3, respectively; and

b) comparing said reacted fraction to a control fraction to determine whether said candidate drug has said activity that promotes cell survival by assaying for an amount of said mammalian BAD, or said fragment, that is phosphorylated at said serine in said reacted fraction as compared to said control fraction.

44. (original) The method of Claim 43, wherein said contacting further comprises contacting said reacted fraction with Bcl-X_L and/or Bcl-2, and said assaying further comprises assaying said reacted fraction for an amount of said mammalian Bcl-X_L/Bcl-2 Associated Cell Death Regulator polypeptide (BAD) bound to Bcl-X_L and/or Bcl-2, or an amount of said fragment bound to Bcl-X_L and/or Bcl-2, respectively.

45. (original) A method of screening a candidate drug for activity that promotes cell survival, said method comprising:

a) preparing a cell culture containing a cell line expressing a mammalian Bcl- X_L/Bcl-2 Associated Cell Death Regulator polypeptide (BAD), or fragment of said mammalian BAD,

1) said mammalian BAD, or said fragment, comprising an amino acid sequence containing a serine at a position corresponding to position 118 of SEQ ID NO:1, position 155 of SEQ ID NO:2, or position 113 of SEQ ID NO:3, said position of said serine being identified by alignment of said amino acid sequence of said mammalian BAD, or said amino acid sequence of said fragment, to SEQ ID NO:1, SEQ ID NO:2, or SEQ ID NO:3, respectively;

2) said cell line having activity that promotes apoptosis, or capable of having activity that promotes apoptosis;

b) contacting said cell culture with said candidate drug to form a reacted fraction;
and

c) comparing cells in said reacted fraction to cells of a control culture in order to determine whether said candidate drug has activity promoting cell survival by monitoring the viability of said cells in said reacted fraction as compared to said cells of a control culture, wherein said cells of a control culture are treated essentially identical to said cell in said reacted fraction, except that said cells of a control culture are not contacted with said candidate drug.

46. (original) The method of Claim 45, wherein said comparing of said cell culture in said reacted fraction to said control cell culture is by:

a) contacting said cells in said reacted fraction with at least one antibody specific for,

- 1) said mammalian Bcl-X_L/Bcl-2 Associated Cell Death Regulator polypeptide (BAD), or said fragment, phosphorylated at said serine, or
- 2) said mammalian BAD, or said fragment, unphosphorylated at said serine; and
- b) assaying for an amount of said antibody binding to said mammalian BAD, or said fragment.

47. (original) A method of inhibiting apoptosis in a cell expressing a mammalian Bcl-X_L/Bcl-2 Associated Cell Death Regulator polypeptide (BAD), or fragment of said mammalian BAD, said method comprising:

- a) preparing a cell culture containing a cell line expressing said mammalian BAD, or said fragment,
 - 1) comprising an amino acid sequence containing a serine at a position corresponding to position 118 of SEQ ID NO:1, position 155 of SEQ ID NO:2, or position 113 of SEQ ID NO:3, said position of said serine being identified by alignment of said amino acid sequence of said mammalian BAD, or said amino acid sequence of said fragment, to SEQ ID NO:1, SEQ ID NO:2, or SEQ ID NO:3, respectively; and
 - b) contacting said cultured cells with an extracellular agent, and/or inducing an intracellular agent, to form a reacted fraction and thereby activating a kinase, in said cells in said reacted fraction, capable of phosphorylating said mammalian BAD, or said fragment, at said serine; and

c) comparing said cultured cells in said reacted fraction to control cells to determine whether apoptosis is inhibited in either said culture cells in said reacted fraction or in said control cells by,

1) assaying for an amount of said mammalian BAD, or said fragment, that is phosphorylated at said serine in said cells in said reacted fraction as compared to said control cells, or

2) monitoring indicia of apoptosis in said cells in said reacted fraction as compared to said control cells,

wherein treatment of said control cells is essentially identical to said cells in said reacted fraction, except that said control cells do not have said mammalian BAD, or said fragment, capable of being phosphorylated by said kinase.

48. (original) The method of Claim 47, wherein said kinase is a cyclic AMP (cAMP)-dependent protein kinase, PKA.

49. (original) The method of Claim 47, wherein said kinase is a heterologous kinase.

50. (original) The method of Claim 47, wherein said mammalian Bcl-X_L/Bcl-2 Associated Cell Death Regulator polypeptide (BAD), is a heterologous mammalian BAD, and said fragment is a fragment of said heterologous mammalian BAD.

51. (original) The method of Claim 47, wherein said extracellular agent and/or said intracellular agent is a ligand of a G-protein-coupled receptor.

52. (original) The method of Claim 51, wherein said ligand is L-epinephrine.

53. (original) A method of assaying a candidate compound for a kinase activity capable of phosphorylating a mammalian Bcl-X_L/Bcl-2 Associated Cell Death Regulator polypeptide (BAD), or fragment of said mammalian BAD, at a position in the amino acid sequence of said mammalian BAD, or at a position in the amino acid sequence of said fragment, corresponding to position 118 of SEQ ID NO:1; position 155 of SEQ ID NO:2; or position 113 of SEQ ID NO:3, respectively, said method comprising:

a) contacting a candidate compound with said mammalian BAD, or said fragment, to form a reacted fraction,

1) said mammalian BAD, or said fragment, comprising an amino acid sequence containing a serine at a position corresponding to position 118 of SEQ ID NO:1, position 155 of SEQ ID NO:2, or position 113 of SEQ ID NO:3, said position of said serine being identified by alignment of said amino acid sequence of said mammalian BAD, or said amino acid sequence of said fragment, to SEQ ID NO:1, SEQ ID NO:2, or SEQ ID NO:3, respectively; and

b) determining whether said candidate compound has kinase activity capable of phosphorylating said mammalian BAD, or said fragment, at said serine by assaying said reacted fraction for an amount of said mammalian BAD, or said fragment, phosphorylated at said serine by said kinase activity.

54. (original) The method of Claim 53, wherein said assaying includes detecting radioactive label on said serine, said radioactive label being attached to said serine when said serine is phosphorylated.

55. (original) The method of Claim 53, wherein said assaying includes detecting a difference in the electrophoretic mobility of said mammalian Bcl-X_L/Bcl-2 Associated Cell Death Regulator polypeptide (BAD), or said fragment, having said serine that is phosphorylated and having said serine that is unphosphorylated.

56. (original) The method of Claim 53, wherein said assaying includes detecting the binding of said mammalian Bcl-X_L/Bcl-2 Associated Cell Death Regulator polypeptide (BAD), or said fragment, to an antibody specific for said mammalian BAD, or said fragment, that is phosphorylated at said serine.

57. (original) The method of Claim 56, wherein said antibody is a monoclonal antibody.

58. (original) A method of screening a candidate drug for activity that promotes the phosphorylation of a mammalian Bcl-X_L/Bcl-2 Associated Cell Death Regulator polypeptide (BAD), or fragment of said mammalian BAD, at a serine at a position in the amino acid sequence of said BAD, or amino acid sequence of said fragment, corresponding to position 118 of SEQ ID NO:1, position 155 of SEQ ID NO:2, or position 113 of SEQ ID NO:3, said position of said serine being identified by alignment of said amino acid sequence of said mammalian BAD, or said amino acid sequence of said fragment, to SEQ ID NO:1, SEQ ID NO:2, or SEQ ID NO:3, respectively, said method comprising:

a) contacting said candidate drug with a sample comprising Bcl-X_L and said mammalian BAD, or said fragment, to form a reacted fraction, said mammalian BAD, or said fragment capable of being phosphorylated at said serine;

b) comparing said reacted fraction to a control fraction to determine whether said candidate drug has activity that promotes said phosphorylation by,

1) assaying for an amount of mammalian BAD, or said fragment, that is not bound to Bcl-X_L or is bound to Bcl-X_L in said reacted fraction as compared to said control fraction, and/or

2) assaying for an amount of said mammalian BAD, or said fragment, that is phosphorylated at said serine in said reacted fraction as compared to said control fraction,

wherein said control fraction is essentially identical to said reacted fraction, except said mammalian BAD, or said fragment, in said control fraction is not contacted with said candidate drug, and/or said control fraction contains said mammalian BAD, or said fragment, not capable of being phosphorylated at said position of said serine.

59. (original) A method of screening a candidate drug for an activity that promotes the phosphorylation of a mammalian Bcl-X_L/Bcl-2 Associated Cell Death Regulator polypeptide (BAD), or fragment of said mammalian BAD, at a serine at a position in the amino acid sequence of said mammalian BAD, or amino acid sequence of said fragment, corresponding to position 118 of SEQ ID NO:1, position 155 of SEQ ID NO:2, or position 113 of SEQ ID NO:3, said position of said serine being identified by alignment of said amino acid sequence of said mammalian BAD, or said amino acid sequence of said fragment, to SEQ ID NO:1, SEQ ID NO:2, or SEQ ID NO:3, respectively, said method comprising:

a) contacting said candidate drug with a sample comprising said mammalian BAD, or said fragment, and a kinase, to form a reacted fraction, said mammalian BAD, or said fragment, capable of being phosphorylated by said kinase; and

b) comparing said reacted fraction to a control fraction to determine whether said candidate drug has activity that promotes said phosphorylation by assaying for an amount of said mammalian BAD, or said fragment, phosphorylated at said serine in said reacted fraction as compared to said control fraction, wherein said control fraction is identical to said reacted fraction, except said control fraction is not contacted with said candidate drug and/or said control fraction contains said mammalian BAD, or said fragment, not capable of being phosphorylated at said position of said serine.

60. (original) A method of screening a candidate drug for activity that promotes phosphorylation, in a cell, of a mammalian Bcl-X_L/Bcl-2 Associated Cell Death Regulator polypeptide (BAD), or fragment of said mammalian BAD, at a serine at a position in the amino acid sequence of said mammalian BAD, or amino acid sequence of said fragment, corresponding to position 118 of SEQ ID NO:1, position 155 of SEQ ID NO:2, or position 113 of SEQ ID NO:3, said position being identified by alignment of said amino acid sequence of said mammalian BAD, or said amino acid sequence of said fragment, to SEQ ID NO:1, SEQ ID NO:2, or SEQ ID NO:3, said method comprising:

a) preparing a culture containing a cell line expressing said mammalian BAD, or said fragment, said cell line having activity that promotes apoptosis, or capable of having activity that promotes apoptosis;

b) contacting said cultured cells with said candidate drug to form a reacted fraction;
and

c) comparing the cells in said reacted fraction to control cells to determine whether said candidate drug has activity that promotes said phosphorylation by,

1) assaying for an amount of said mammalian BAD, or said fragment, phosphorylated at said serine in the cells in said reacted fraction as compared to said control cells, or

2) monitoring indicia of apoptosis in the cells in said reacted fraction as compared to said control cells,

wherein said control cells are identical to the cells in said reacted fraction, except said control cells are not contacted with said candidate drug.

61. (original) The method of Claim 60, wherein said assaying further comprises contacting said cell with at least one antibody, said antibody being selected from a group consisting of at least one antibody specific for said mammalian BAD, or said fragment, that is phosphorylated at said serine, or at least one antibody specific for said mammalian BAD, or said fragment, that is unphosphorylated at said serine.

62. (original) A method of screening a candidate drug for activity that modulates apoptosis promoting activity in a cell, said method comprising:

a) preparing a culture containing a cell line expressing mammalian Bcl-X_L/Bcl-2 Associated Cell Death Regulator polypeptide (BAD), or fragment of said mammalian BAD,

1) said mammalian BAD, or said fragment, comprising an amino acid sequence containing a serine at a position corresponding to position 118 of SEQ ID NO:1, position 155 of SEQ ID NO:2, or position 113 of SEQ ID NO:3, said position of said serine being identified by

alignment of said amino acid sequence of said mammalian BAD, or said amino acid sequence of said fragment, to SEQ ID NO:1, SEQ ID NO:2 or SEQ ID NO:3, respectively;

b) contacting said cultured cells with an apoptosis promoting substance, wherein said cultured cells have activity that promotes apoptosis, or is capable of having activity that promotes apoptosis;

c) contacting said cultured cells with said candidate drug to form a reacted fraction;
and

d) comparing the cells in said reacted fraction to control cells to determine whether said candidate drug has activity that modulates apoptosis promoting activity by,

1) determining the amount of said mammalian BAD, or said fragment, that is phosphorylated or unphosphorylated at said serine in the cells in said reacted fraction as compared to said control cells, or

2) monitoring indicia of apoptosis in the cells in said reacted fraction as compared to said control cells,

wherein said control cells are identical to the cells in said reacted fraction, except that said control cells are not contacted with said candidate drug.

63-69. (canceled).

70. (new): The mutant BAD or fragment of mutant BAD of claim 16, wherein said alanine enhances the *in vitro* cell death promoting activity of said mutant BAD or fragment of mutant BAD.